

(d) a cloned polynucleotide of interest linked to a first inducible promoter,

wherein in said gene expression system, the expression product of the IF gene, or functional analogue thereof

(i) induces the production of bacteriocins by a lactic acid bacterium,

(ii) is not a lantibiotic, and

(iii) induces the expression of genes regulating bacteriocin production in said lactic acid bacterium, and

(iv) activates the expression product of the SakK gene, or functional analogue thereof, and

the activated expression product of the SakK gene, or functional analogue thereof, activates the expression product of the SakR gene, or functional analogue thereof, and

the activated expression product of the SakR gene, or functional analogue thereof, induces the first promoter of the gene of interest,

thereby causing expression of the gene of interest; and

wherein the IF gene or functional analogue thereof is expressed from a promoter different from the promoter from which the SakK gene or functional analogue thereof and/or the SakR gene or functional analogue thereof are expressed.

70. A gene expression system comprising:

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- (a) an IF gene;
  - (b) a SakK gene;
  - (c) a SakR gene;
  - (d) a cloned polynucleotide of interest linked to a first inducible promoter,

wherein in said gene expression system, the expression product of the IF gene activates the expression product of the SakK gene, and

the activated expression product of the SakK gene activates the expression product of the SakR gene and

the activated expression product of the SakR gene induces the first promoter of the gene of interest,

thereby causing expression of the gene of interest;

wherein said the expression product of said IF gene is not a lantibiotic; and

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wherein the IF gene or functional analogue thereof is expressed from a promoter different from the promoter from which the SakK gene or functional analogue thereof and/or the SakR gene or functional analogue thereof are expressed.

71. The gene expression system of claim 69, wherein said expression product of the IF gene, or a functional analogue thereof, comprises the amino acid sequence of residues 19-37 of SEQ ID NO:3.

72. The gene expression system of claim 70, wherein said expression product of an IF gene has the amino acid sequence of residues 19-37 of SEQ ID NO:3.

73. The gene expression system of claim 69, wherein the SakK gene, or functional analog thereof, and the SakR gene, or functional analog thereof, are operably linked to a constitutive promoter.

74. The gene expression system of claim 73, wherein the first inducible promoter and gene of interest are on a first vector and the SakK gene, or functional analog thereof, and the SakR gene, or functional analog thereof are operably linked to a constitutive promoter located on a second vector separate from the first vector.

75. The gene expression system of claim 69, wherein the SakK gene, or functional analog thereof, and the SakR gene, or functional analog thereof, are operably linked to a second inducible promoter.

76. The gene expression system of claim 75, wherein the first inducible promoter and gene of interest are on a first

vector and SakK gene, or functional analog thereof, and the SakR gene, or functional analog thereof, are operably linked to a second inducible promoter located on a second vector.

77. The gene expression system of claim 69, wherein a functional analogue of the IF gene is a plnA gene, a functional analogue of the SakK gene is a plnB gene, a functional analogue of the SakR gene is a plnC gene or a plnD gene,

wherein plnA, plnB, plnC, and plnD are genes of a lactic acid bacterium.

78. A host cell comprising the gene expression system of claims 69 or 70.

79. The host cell of claim 78, wherein the host cell is a Gram-positive bacterium.

80. The host cell of claim 79, wherein the host cell is a lactic acid bacterium.

81. The host cell of claim 80, wherein the host cell is selected from the group of genera consisting of *Lactobacillus*, *Lactococcus*, and *Pediococcus*.

82. The host cell of claim 81, wherein the host cell is *Lactobacillus sake* or *Lactobacillus plantarum*.

83. The host cell of claim 82, wherein said host cell is *Lactobacillus sake* LTH673 or *Lactobacillus plantarum* C11.

84. The gene expression system of claim 69, wherein the SakK gene, or functional analog thereof, and the SakR gene, or functional analog thereof, are transcribed in an operon.

85. The gene expression system of claim 70, wherein the the SakK gene and the SakR gene are transcribed in an operon.

86. The gene expression system of claim 69, wherein the first inducible promoter comprises two repeated nucleotide sequences 5 to 10 nucleotides long and spaced 17 to 23 nucleotides apart, wherein the downstream member of said repeated sequence is located 30 to 38 nucleotides downstream from a -10 region of a bacterial gene, and wherein said repeated nucleotide sequences are selected from the group consisting of residues 7-14 and 30-38 of SEQ ID NO:6, residues 7-14 and 30-38 of SEQ ID NO:7, residues 7-14 and 30-38 of SEQ ID NO:8, residues 7-14 and 31-38 of SEQ ID NO:9, residues 7-8, 10-14 and 31-38 of SEQ ID NO:10.--

87. A method for producing a protein of interest comprising:

culturing a host cell comprising the gene expression system of claim 69 in a medium, wherein expression of the gene of interest produces the protein of interest; and

purifying the protein of interest from the culture.

88. A method for producing a protein of interest comprising:

culturing a host cell comprising the gene expression system of claim 70 in a medium, wherein expression of the gene of interest produces the protein of interest; and

purifying the protein of interest from the culture.

89. A kit for a gene expression system, comprising:

a) at least one vector comprising (i) a promoter that can be induced by the expression product of a SakR gene or a functional analog thereof, wherein the SakR expression product is activated by the expression product of a SakK gene or a functional analog thereof, wherein the SakK expression product is activated by the expression product of an IF gene or a functional analog thereof; (ii) a cloning site, and (iii) at least one gene selected from the group consisting of a SakK

gene, a SakR gene, an IF gene, or a functional analogue of said genes; and

b) a host strain having a chromosome comprising at least one gene not present on the vector a) and selected from the group consisting of a SakK gene, a SakR gene, an IF gene, or a functional analogue of said genes.

90. A kit for gene expression comprising:

a) at least one vector comprising (i) a promoter that can be induced by the expression product of a SakR gene of a lactic acid bacterium, wherein the SakR expression product is activated by the expression product of a SakK gene of a lactic acid bacterium, wherein the SakK expression product is activated by the expression product of an IF gene of a lactic acid bacterium; and (ii) a cloning site; and

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b) a host strain having a chromosome comprising a SakK gene of a lactic acid bacterium and a SakR gene of a lactic acid bacterium.

91. A kit for a gene expression system, comprising:

a) at least one vector comprising (i) a promoter that can be induced by the expression product of a SakR gene or a functional analog thereof, wherein the SakR expression product is activated by the expression product of a SakK gene or a

functional analog thereof, wherein the SakK expression product is activated by the expression product of an IF gene or a functional analog thereof and (ii) a cloning site,

b) a SakK gene of a lactic acid bacterium and a SakR gene of a lactic acid bacterium, wherein the SakK gene and the Sak R gene are each independently present either on the vector a) or on a second, separate vector.

92. The kit of claim 90, further comprising c) a peptide comprising the amino acid sequence of residues 19-37 of SEQ ID NO:3, or an analog thereof that can activate the expression product of a SakK gene or a functional analog of a SakK gene.

93. The kit of claim 91, further comprising b) a peptide comprising the amino acid sequence of residues 19-37 of SEQ ID NO:3, or an analog thereof that can activate the expression product of a SakK gene of a lactic acid bacterium.

94. A gene expression system comprising:

- (a) an IF peptide, or a functional analogue thereof;
- (b) a SakK gene, or a functional analogue thereof;
- (c) a SakR gene, or a functional analogue thereof;

(d) a cloned polynucleotide of interest linked to a first inducible promoter,



wherein in said gene expression system, the IF peptide, or functional analogue thereof activates the expression product of the SakK gene, or functional analogue thereof, and

the activated expression product of the SakK gene, or functional analogue thereof, activates the expression product of the SakR gene, or functional analogue thereof, and

the activated expression product of the SakR gene, or functional analogue thereof, induces the first promoter of the gene of interest;

thereby causing expression of the gene of interest;

wherein the first inducible promoter is a promoter different from the promoter from which the SakK gene or functional analogue thereof and/or the SakR gene or functional analogue thereof are expressed.

95. A gene expression system comprising:

(a) an IF peptide;

(b) a SakK gene;

(c) a SakR gene;

(d) a cloned polynucleotide of interest linked to a first inducible promoter,

wherein in said gene expression system, the the IF peptide activates the expression product of the SakK gene, and

the activated expression product of the SakK gene activates the expression product of the SakR gene and

the activated expression product of the SakR gene induces the first promoter of the gene of interest,

thereby causing expression of the gene of interest;

wherein the first inducible promoter is a promoter different from the promoter from which the SakK gene or functional analogue thereof and/or the SakR gene or functional analogue thereof are expressed.

96. The gene expression system of claim 94, wherein the IF peptide, or a functional analogue thereof, comprises the amino acid sequence of residues 19-37 of SEQ ID NO:3.

97. The gene expression system of claim 95, wherein the IF peptide has the amino acid sequence of residues 19-37 of SEQ ID NO:3.

98. The gene expression system of claim 94, wherein the SakK gene, or functional analog thereof, and the SakR gene, or functional analog thereof, are operably linked to a constitutive promoter.

99. The gene expression system of claim 95, wherein the first inducible promoter and gene of interest are on a first vector and the SakK gene, or functional analog thereof, and the SakR gene, or functional analog thereof are operably linked to a constitutive promoter located on a second vector separate from the first vector.

100. The gene expression system of claim 94, wherein the SakK gene, or functional analog thereof, and the SakR gene, or functional analog thereof, are operably linked to a second inducible promoter.

101. The gene expression system of claim 94, wherein the first inducible promoter and gene of interest are on a first vector and SakK gene, or functional analog thereof, and the SakR gene, or functional analog thereof, are operably linked to a second inducible promoter located on a second vector.

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102. The gene expression system of claim 94, wherein a functional analogue of the SakK gene is a plnB gene, a functional analogue of the SakR gene is a plnC gene or a plnD gene, wherein plnB, plnC, and plnD are genes of a lactic acid bacterium.

103. A host cell comprising parts b), c) and d) of the gene expression system of claims 94 or 95.

104. The host cell of claim 103, wherein the host cell is a Gram-positive bacterium.

105. The host cell of claim 103, wherein the host cell is a lactic acid bacterium.

106. A method for producing a polypeptide or protein of interest comprising culturing a host cell comprising the gene expression system of claim 103 in a medium, adding an IF peptide, or functional analog thereof, to the culture, thereby inducing expression of the gene of interest to produce the polypeptide or protein of interest; and

purifying the protein of interest from the culture.

107. An isolated nucleic acid comprising:

two repeated nucleotide sequences 5 to 10 nucleotides long and spaced 17 to 23 nucleotides apart, wherein the downstream member of said repeated sequence is located 30 to 38 nucleotides downstream from a -10 region of a bacterial gene,

wherein transcription of a coding nucleic acid sequence operatively linked to said isolated nucleic acid is activated by

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an expression product of a SakR gene or functional analog thereof that has been activated by an expression product of a SakK gene or functional analog thereof.

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108. The isolated nucleic acid of claim 107, wherein said repeated nucleotide sequences are selected from the group consisting of residues 7-14 and 30-38 of SEQ ID NO:6, residues 7-14 and 30-38 of SEQ ID NO:7, residues 7-14 and 30-38 of SEQ ID NO:8, residues 7-14 and 31-38 of SEQ ID NO:9, residues 7-8, 10-14 and 31-38 of SEQ ID NO:10.--